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COMPARATIVE BIOMONITORING OF RAW LEACHATES FROM SOME URBAN WASTE DUMPSITES USING *Allium cepa* ASSAY

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ABSTRACT
Leachates from solid wastes have been implicated in the pollution of the environment including surface and groundwater contamination which ultimately affect human health. Raw leachates were obtained from three open dumpsites: a University campus (CSL), an urban market place (UML), and a domestic dumpsite (OSL), and were evaluated for their possible genotoxic effects on the root tip cells of *Allium cepa* L. The leachates were examined for the presence of heavy metals using standard methods. The onion bulbs were cultivated in 1%, 2.5%, 5% and 20% of each leachate sample, and after 48 hours, one root tip from each bulb was harvested and processed for cytological observation using the aceto-orcein squash technique. After 72 hours, mean root lengths of the *Allium cepa* were obtained. The results revealed that compared to the control, treatment with the leachates resulted in significant ($p<0.05$) concentration-dependent onion root growth inhibition with EC_{50} values of 3.2%, 4.5%, and 5.7% for CSL, UML and OSL respectively. The leachates were characterized by various chromosomal aberrations and reduction in the number of dividing cells. The physical and chemical properties showed that the leachates were acidic and contained toxic chemicals. CSL was most toxic, while OSL was least toxic. The results obtained suggest that the toxic chemicals present in these leachates pose a threat to the environment and human health if left unchecked.

KEYWORDS: *leachates, genotoxicity, Allium cepa, chromosomal aberrations*

INTRODUCTION
Leachates and/or runoffs from open dumps which do not have concrete walls percolate freely into the soils thereby contaminating adjoining surface and ground water which form the major sources of drinking water and usually, these water sources are abstracted without any form of further treatment. Exposure to multiple chemical combinations in population living near waste dump sites has led to series of human health disorders (Vrijheid 2000, Palmer *et al*., 2005). The impact of leachates from unsecured dumpsites which have been implicated in environmental pollution and specifically on the surface and groundwater has given rise to a number of studies in recent years (Nubi *et al.*, 2008, Alkassasbeh *et al*., 2009, Adewuyi and Opasina 2010).

Many workers have successfully employed the *Allium cepa* assay, a plant bioassay, as a rapid bio-tool for genotoxicity screening of leachates in the South-West of Nigeria (Odeigah *et al*., 1997, Bakare *et al*., 2000, Bakare 2001, Bakare and Wale-Adeyemo 2004). We are not aware of any studies conducted to characterize leachates from open dumpsites in the Mid-West, particularly in Benin City. The objective of this study therefore, was to the evaluate the genotoxic potential of leachates from selected open dump sites in Benin City by employing root meristem cells of *A. cepa* L.

MATERIALS AND METHODS
Sampling Sites
Raw leachates were collected in September, 2010 from leachate wells from three dump sites in Uselu, Ugbowo, and Oluku in the Benin metropolis in Edo State situated between latitudes 6° 06' N, 6° 30' N and longitudes 5° 30' E, 5° 45' E. The dump site in Uselu is located in the market while the Ugbowo open dump is at the *Capitol* in the University of Benin, Ugbowo Campus premises. The Oluku dump site is located on the Benin-Akure Road.

Sampling and Leachate Collection
Raw leachates were collected from different spots at the three open dump sites (not less than 25 grabs) in a pre-washed 25 L plastic container, so as to have a composite sample and stored at 4°C. The samples are designated as follows: University of Benin Ugbowo campus leachate (CSL), Uselu market leachate (UML), and the Oluku

dumpsite leachate (OSL). The raw leachates were filtered with a sieve to remove debris. Tap water of good quality was used to dilute the leachates to obtain four concentrations (1%, 2.5%, 5% and 20%) respectively.

Allium cepa Assay

Onion bulbs *(Allium cepa* L., 2n=16*)* of the purple variety of average size (15-22 mm diameter) were purchased locally in Benin City, Edo State, Nigeria. They were sun-dried for six weeks and the dried roots present at the base of the onion bulbs were carefully shaved off with a sharp razor blade to expose the fresh meristematic tissues. The bulbs were then placed in freshly prepared distilled water to protect the primordial cells from drying up. To account for a number of bulbs in the population that would be naturally slow or poor growing, seven replicate bulbs were used for each test sample and control (tap water) and the best five bulbs were chosen for examination (Rank and Nielsen 1993). The bulbs were removed from the distilled water and placed on a blotting paper to remove excess water.

For root growth inhibition evaluation, the bulbs were exposed directly in 1, 2.5, 5, and 20% (v/v, leachate/tap water) of each of the test samples. The highest concentration selected was 20% because there were no root growth at all other higher concentrations. Seven onion bulbs were utilized for each concentration of each leachate and the control. Tap water of good quality was used for the control and the experiment was performed in the dark at 25±1°C. The base of each of the bulbs was suspended on the leachate sample inside 100ml beakers for 72 h. Test effluents were changed daily. At the end of the exposure period, the roots of five onion bulbs with the best growth at each concentration were removed with a forceps and their lengths measured (in cm) with a metre rule. The effect of each sample on the morphology of growing roots was also examined.

For the evaluation of induction of chromosomal aberration, root tips from these bulbs were cut and fixed in ethanol:glacial acetic acid (3:1, v/v) at the end of 48 h. These were hydrolyzed in 1N HCl at 60°C for five minutes after which they were washed in distilled water. Two root tips were then squashed on each slide, stained with aceto-carmine for 10 min and cover slips carefully lowered on it to exclude air bubbles. The cover slips were sealed on the slides with clear fingernail polish as suggested by Grant (1982). This is to prevent drying out of the preparation by the heat of the microscope (Sharma 1983). Six slides were prepared for each concentration and the control, out of which five were analyzed at ×1000 magnification for induction of chromosomal aberrations. The mitotic index was calculated as the number of dividing cells per 1000 observed cells and the frequency of aberrant cells (%) was calculated based on the number of aberrant cells per total cells scored at each concentration of each leachate as in previous studies (Olorunfemi *et al.*, 2011).

The leachates were analyzed for a number of standard physico-chemical properties, including dissolved oxygen (DO), total dissolved solids (TDS), biochemical oxygen demand (BOD), chlorides, nitrates, and phosphates, according to methods described by APHA (1998). Nine metals (including seven heavy metals) namely: lead, cadmium, copper, iron, zinc, nickel, and manganese, were analyzed in the samples according to standard analytical methods (USEPA 1996, APHA 1998).

The means, with 95% confidence limits and the standard errors for results of the root inhibition and chromosome aberrations of each concentration of the wastewaters were calculated. Data were expressed as Mean ± Standard Error of Mean (SEM). Differences between the control and the different concentrations of the wastewaters were analyzed by means of the Student's unpaired *t*-test. P values of <0.05 were considered to be statistically significant. All statistical analyses were carried out using SPSS®14.0 statistical package.

Results and Discussion

Raw leachates from domestic wastes in open dump sites and municipal landfills in South-West Nigeria have been recognized as sources of chemical constituents (Bakare *et al.,* 2000, Bakare 2001, Alimba *et al.,* 2006), a mix of which may result in synergistic combinations that are more harmful than individual leachate chemical in living systems (Bakare and Wale-Adeyemo 2004). Results from the physical and chemical analyses of the raw leachate samples used in this study (Table 1) are in agreement with these studies. Compared to the permissible limits set by regulatory bodies (FEPA 1991, USEPA 1999, WHO 1996), most of the parameters analysed in these leachates were present at appreciably high concentrations.

The types of wastes, solid and liquid from the waste dumps differed from each other. The wastes from the market were mostly decomposing food items, agricultural and domestic materials while the dump site in the

University campus contained such materials as sanitary pads, papers, computer consumables, plastics and metal cans. The solid dumps at Oluku were mostly household wastes and papers. The values obtained from the analyses was a reflection of the type of materials found in the dumps, for instance, the heavy metals (Cu, Fe, Zn, Al, Ni, Cd and Cr) were comparatively highest in leachates from Capitol dump site. Similarly, total dissolved solids, total alkalinity, chlorides, sulphates and nitrates were also highest in Capitol leachate. The pH of capitol leachate was 3.9, while Uselu leacheate had a pH of 4.1.

Table 1: Physico-chemical characteristics of raw leachates obtained from Capitol, Uselu and Oluku open dump sites.

Parameter	CSL	UML	OSL	FEPA[a]	USEPA[b]	WHO[c]
pH	4.1	3.9	6.1	6-9	6.5-8.5	6.5-9.5
BOD_5 @ 20^{o}C	6.7	7.4	5.6	50	NS	NS
TDS	0.08	0.07	0.09	2000	500	<1200
Total Alkalinity	5.7	6.35	3.25	NS	NS	NS
Chlorides	754.78	937.91	674.5	600	250	250
Sulphates	136.5	149	128.5	500	250	500
Nitrates	39.5	37.5	33.5	20	10	50
Nickel	0.3	1.1	0.3	<1	0.005	0.02
Copper	2.6	5.9	1.9	<1	0.009	NS
Iron	2.6	5.9	7.1	20	0.30	NS
Cadmium	11.3	8.1	0.1	<1	0.002	0.003
Mercury	ND	ND	ND	0.05	NS	NS
Manganese	1.8	1.5	1.1	5	0.05	NS
Zinc	33.9	55.3	31.1	<1	0.12	0.01
Aluminum	0.4	0.5	0.3	NS	NS	NS
Chromium	0.9	1.6	0.3	0.05	NS	0.05

Values are means of 3 replicates
All values are in mg/l except Temperature (°C), Colour (TCU), Turbidity (NTU) and pH with no unit.
BOD: Biochemical oxygen demand, TDS: Total dissolved solids, [a]Federal Environmental Protection Agency (1991) Permissible limits for effluent discharge into surface water, [b]United States Environmental Protection Agency (1999).National recommended water quality criteria correction, [c]World Health Organisation (1996). Guideline for drinking water quality recommendation, NS: Not stated, ND: Not detected

The mean root lengths of *Allium cepa* grown in Capitol, Uselu and Oluku leachates at different concentrations are shown in Tables 2. There was a gradual inhibition of root growth in all the leachates with increasing concentration. The descending order of decrease of mean root length at all concentrations was CSL>UML>OSL. The EC_{50} values were 3.2%, 4.5%, and 5.7% for Capitol, Uselu and Oluku leachates respectively (Fig. 1).

Table 2: Root length of *Allium cepa* after cultivation in different concentrations of raw leachates obtained from Capitol, Uselu and Oluku open dump sites.

Conc (%)	CSL			UML			OSL		
	Mean Root length±S.E. (cm)	RG(%) of control	95% CL	Mean Root length±S.E. (cm)	RG(%) of control	95% CL	Mean Root length±S.E. (cm)	RG(%) of control	95% CL
0	3.90±0.64	100	44	3.90±0.64	100	44	3.90±0.17	100	44
1	2.30±0.48	93.3	38	2. 90±0.17	93.3	38	3.01±0.30	92.8	38
2.5	1.90±0.32	63.3	54	2.70±0.43	86.6	41	2.96±0.29	92.0	40
5	1.76±0.04	58.6	41	2.20±0.25	86.6	54	2.60±0.08	85.7	23
20	1.00±0.23	40	34	2.00±0.19	64.55	20	2.230±0.32	64.2	13
EC_{50}	3.2%			4.7%			5.7%		

RG (%) of control expressed as % root growth of the control.
95% CL: 95% confidence limit.
* $P < 0.05$, level of significance of root growth inhibition compared with the untreated control.
p values, (> 0.05) level as compared to controls, Values are Mean ± SEM

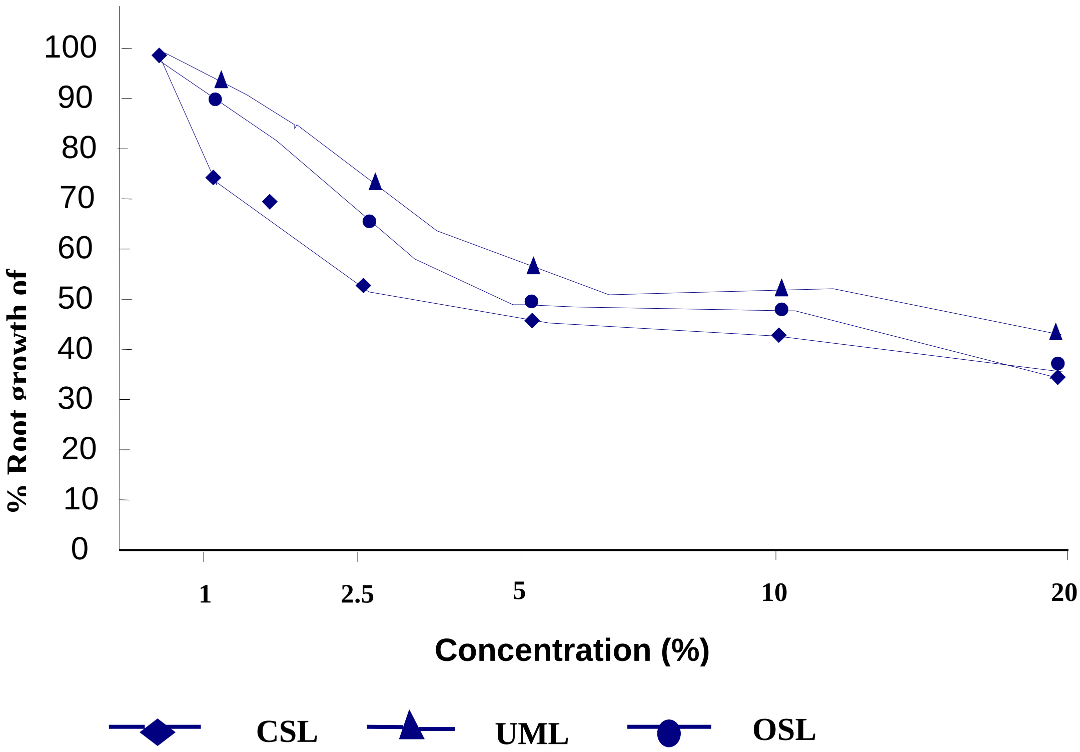

Fig. 1: Effects of raw leachates from urban dump sites in Benin City on root growth of *A. cepa*

Mitotic index (MI) is used as a bio-monitor to assess the mutagenicity of effluents (Fiskesjö 1985). Results of the microscopic analysis of the treated *Allium cepa* root tips are summarised in Table 3. Chromosomal aberrations were induced at all the tested concentrations of the leachates and were statistically significant (p<0.05). Compared to the negative control, there was lower mitotic index and increase in chromosomal aberrations at all concentrations; a phenomenon conforming to reports of Bakare *et al*. (2000) and Bakare and Wale-Adeyemo (2004) in landfill leachates. This may probably be due to the increased values of heavy metals as leachate concentration increased. The cytotoxicity level may be determined by the degree of the mitotic index (Panda and Sahu 1985); a mitotic index decrease below 22% of the control causes lethal effects while MI

below 50% of the control has sublethal effects (Antonsie-Wiez 1990). These results indicate that the leachates could inflict lethal and sublethal effects in living systems in their undiluted and 1% leachate concentration.

Table 3: Cytological effects of raw leachates obtained from Capitol, Uselu and Oluku open dump sites on cells of *Allium cepa* L.

*5000 cells per conc. of each effluent and the control

Leachate	Conc (%)	Mitotic index (%±SE)+	Stickiness (%±SE)+	Bridges (%±SE)+	Fragments (%±SE)+	Bi-nucleated cells (%±SE)+	Laggards (%±SE)+	Spindle disturbance (%±SE)+	Aberrant cells (%)+
Control	0	30.03±2.16	0	0.33±0.08	0	0	1.00±0.17	0.98±0.06	2.31±0.89
	1	16.16±1.03	4.67±1.03	1.33±1.03	5.60±2.06	1.00±0.58	3.50±1.61	3.76±1.12	19.86
CSL	2.5	10.04±2.16	6.67±0.81	2.50±0.54	6.34±1.01	4.17±0.60	3.83±1.12	4.83±0.75	28.34
	5	8.07±1.02	8.33±1.31	3.31±0.78	7.02±1.31	4.83±0.73	4.52±1.05	6.16±1.11	34.17
	20	4.16±1.12	10.01±1.14	4.34±0.75	11.31±0.67	5.01±0.98	4.91±1.12	7.50±0.78	43.08
	1	25.28±3.61	3.17±0.41	1.50±0.81	2.50±0.54	0	2.16±0.98	3.83±0.75	13.16
UML	2.5	19.67±1.78	4.50±1.41	1.67±1.02	3.83±1.13	0.78±0.03	2.67±1.11	4.00±1.56	17.45
	5	16.01±3.80	5.17±1.17	2.51±1.20	4.67±1.31	1.33±0.43	3.17±1.11	4.33±1.21	21.18
	20	10.03±1.20	5.00±0.98	2.17±1.41	3.83±1.46	1.98±0.78	3.10±0.98	4.17±0.78	20.25
	1	18.33±3.66	2.03±0.10	0.78±0.11	1.50±0.54	0	2.00±0.08	2.08±0.35	8.39
OSL	2.5	16.25±1.71	3.10±1.02	1.00±0.08	1.83±1.13	0	2.17±0.02	3.06±1.06	11.16
	5	15.10±1.63	3.97±1.11	1.51±0.98	2.67±1.31	0	2.78±0.60	2.33±1.01	13.26
	20	13.25±3.67	3.98±1.00	2.01±0.64	2.86±0.96	1.00±0.63	2.88±0.46	3.02±0.96	15.75

+Significant difference from control at P<0.05

Chromosomal anomalies have been found to highly correlate to mutagenic events. The most frequent aberrations observed in this study were sticky chromosomes, laggards, chromosome breaks and spindle disturbance and were similar to those reported by Bakare and Wale-Adeyemo (2004) indicating that the leachates contained constituents that are cytotoxic and could induce mutation in *A. cepa*. There was a linear relationship between the genotoxicity parameters and root length inhibition. This is an indication that the leachates especially at high concentrations are capable of changes in chromosome structure and number. The results of physico-chemical analyses corroborate this assertion. The fact that CSL had the highest % aberrant cells at all concentrations suggests that it is the most toxic of the leachates. Conversely, OSL with the least percentage of these aberrant chromosomes suggests that it is the least toxic.

The results imply that the leachates could result in groundwater contamination even at dilute concentrations; moreover exposure to leachate pollution in groundwater might lead to the damage of cytogenetic material of living organisms *in vivo*. The leachates used in this study were raw. Simulated leachates have been found to be more toxic than raw leachates (Bakare and Wale-Adeyemo 2004). Already, the USL raw leachates are obviously responsible for the foul odour emanating from the dump site in Uselu market. This is worrisome considering the fact that the market is situated right at the heart of residential houses, many of which have sunk boreholes as their source of drinking water. CSL open dump in the University of Benin, Ugbowo campus, produces leachates which are directly washed into the Ikpoba river while OSL leachates would probably leak

into the nearby Iguosa river. The urgent need for proper environmental management by relevant authorities to avert possible detrimental consequences of these leachates on plant and animal life cannot be overemphasized.

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